Enzymatic biodiesel synthesis using novel process intensification principles

Ilmi, Miftahul

CHAPTER 1

Introduction

1. Fossil resources and possible alternatives

In the last century global energy consumption showed a rapid increase and actually doubled from 25.5×10^{10} Gigajoules in 1973 to 56.7×10^{10} Gigajoules in 2013. The consumption is expected to increase further, potentially up to 34% between 2014 and 2035 [1,2]. Fossil fuels (81.4%) are the main source for energy, with a main share from petroleum (31%) [1]. Besides the energy sector, the chemical industry is also heavily dependent on petroleum. Over 90% (by tonnage) of all organic chemicals are derived from it [3]. Examples of base chemical produced from petroleum are ethylene, propylene, C4-olefines, and BTX (the aromatics benzene, toluene and xylene) [4].

However, the limited availability of fossil fuels is a major concern, and for instance by the end of 2014, the oil reserves were estimated to be depleted in about 52 year [5]. This grim picture is a major driving force for the development of alternative renewable resources. In addition, fossil fuels are the main source of Green House Gas (GHG) emissions, which are expected to have a major impact on our global climate. The concentration of CO_2 in the atmosphere has increased with 1.9 ppm per year in the period 1995 to 2005 [6]. It is predicted that the global average concentration of CO_2 will increase rapidly from 379 ppm in 2005 till 730 - 1020 ppm in 2100 [6].

Renewable energy resources are available on large scale; examples are hydropower, wind, solar, biomass, and geothermal energy (Figure 1.1) [7]. Of all renewable energy sources, solar energy for electricity generation has shown the highest growth rate in recent years [7]. Biomass is the only renewable resource that can provide green carbon for transportation fuels and chemicals [8]. Substitution of fossil fuels with biofuels will have a large positive effect on CO_2 emission, and in combination with CO_2 capture and storage even carbon negative results can be obtained [9,10]. Replacement of 2% of the fossil fuels with biofuels is predicted to give a 1.8% reduction in CO_2 emissions, while a 100% replacement will lead to a reduction of 90% [11].

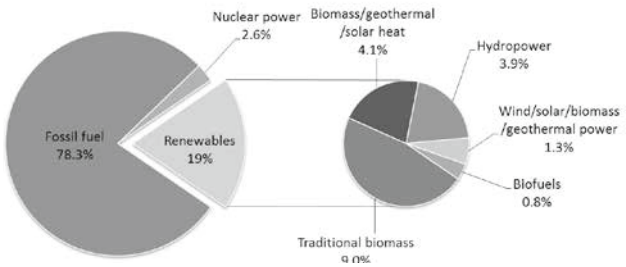


Figure 1.1. Global energy consumption in 2013. Data from [7]

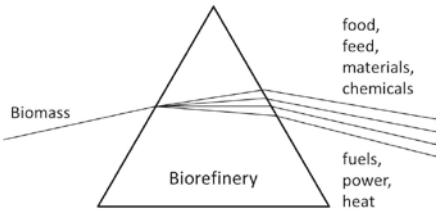


Figure 1.2. A biorefinery in action. Redrawn from [15].

Table 1.1. Typical biomass feedstocks for biorefineries including recycle times and productivities [16].

Feedstock	Recycle time	Biomass yield[a] tons/ha	Biomass production tons/(ha year)
Algae	1 month	9.0	11.25
Agricultural crops	3 month – 1 year	4.5	2.93
Temperate grasses	1 year	7.2	2.70
Savannah	1 year	18.0	4.05
Shrubs	1 – 5 years	27.0	3.15
Tropical forest	5 – 25 years	202.5	9.90
Tropical season forest	5 – 25 years	157.5	7.20
Boreal forest	25 – 80 years	90.0	3.60
Temperate deciduous	10 – 50 years	135.0	5.40
Temperate evergreen	10 – 80 years	157.5	5.85
Oil, gas and coal	280 million years	$(38.4 \times 10^{27}$ J)	(0)

[a]amount of biomass on a mass basis per unit surface area

Recently, the term bio-based economy has been introduced [12,13]. The term encapsulates a vision of a future society that no longer is wholly dependent on fossil fuels for energy and industrial raw materials and preferably uses renewable feedstocks like biomass. The European Commission published a document entitled "Strategy for a sustainable bio-economy to ensure smart green growth in Europe" in February 2012 promoting a more innovative, low-emission economy which reconciles demands for sustainable agriculture and fisheries, food security and the sustainable use of renewable biological resources for industrial purposes, while also ensuring biodiversity and environmental protection [14].

2. Biorefinery concepts

An important valorisation concept in the bio-based economy involves the use of biorefineries. According to the International Energy Agency (IEA) Bioenergy Task 42, biorefinering is the sustainable processing of biomass into a spectrum of marketable products and energy (Figure 1.2). This means that a biorefinery

can be a facility, a process, a plant, or even a cluster of facilities [15]. Individual conversion processes in biorefineries are biomass pretreatment, thermochemical conversions, chemical conversions, enzymatic conversions, and microbial conversions [8,15]. The biomass input in a biorefinery can be from dedicated crops, residues or wastes from industries and households, woody biomass, and aquatic biomass. Examples of typical biomass feeds are given in Table 1.1. The biomass is subsequently converted into both intermediates and final products (food, feed, materials, chemicals) which are marketable; and energy such as fuels, power, and heat [8,15].

3. Biofuels

Transportation fuels like gasoline and diesel are mostly derived from crude oil and as such the transportation sector is a major contributor to CO_2 emissions (33.6% in 2013) [1]. It is predicted that the consumption of liquid fuels in the transportation sector will increase by an average of 1.1% per year [17]. The limited oil reserves [5] and environmental issues [6] have been a global driver to develop biofuels.

Plant derived biomass is an important source for biofuels Commercially available biofuels (bioethanol and biodiesel) are produced from edible agricultural crops, such as cereals, sugar crops, and oil seeds. These biofuels are generally referred to as first generation biofuels [18,19]. The main first generation biofuel is bioethanol, of which about 80% is produced from sugarcane and corn. For biodiesel production, edible vegetable oils, such as sunflower and palm oil, are used [19]. First generation biofuels are currently produced commercially in large scale facilities.

However, first generation biofuels compete with the food sector for input [19,20]. This has spurred the development of the use of non-edible feedstock for biofuel production. Particularly ligno-cellulosic biomass, including agricultural by-products and waste, and non-edible plant oils have attracted large attention [20]. Biofuels derived from these feedstocks are designated as second generation biofuels [19]. Technologies for the production of second generation biofuels are typically more complex than for first generation biofuels. For instance, for the production of second generation bioethanol from woody biomass an additional saccharification steps is required [19,20]. In Figure 1.3 some relevant examples of first and second generation biofuels including the major processing steps are provided.

Alternative biomass feedstocks for biofuel production are receiving large attention at the moment, and a well-known example is the use of algae [19,21].

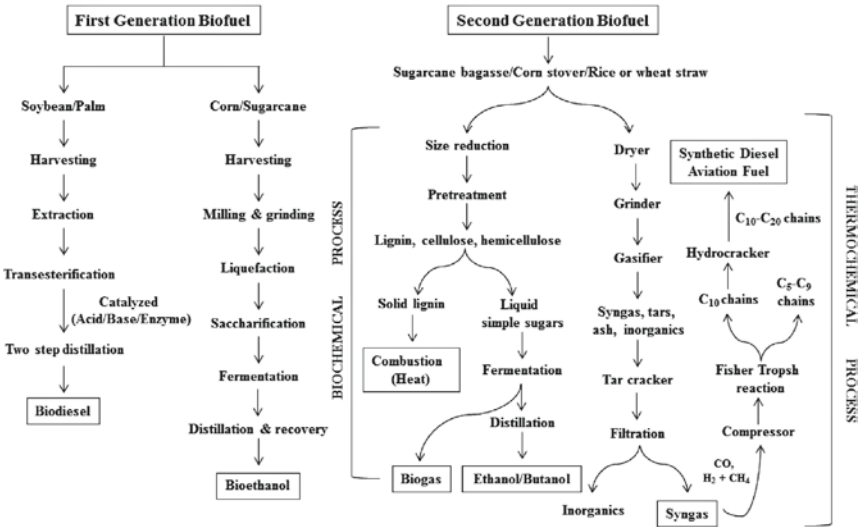


Figure 1.3. Examples of first and second generation biofuels. Reproduced from [19] with permission.

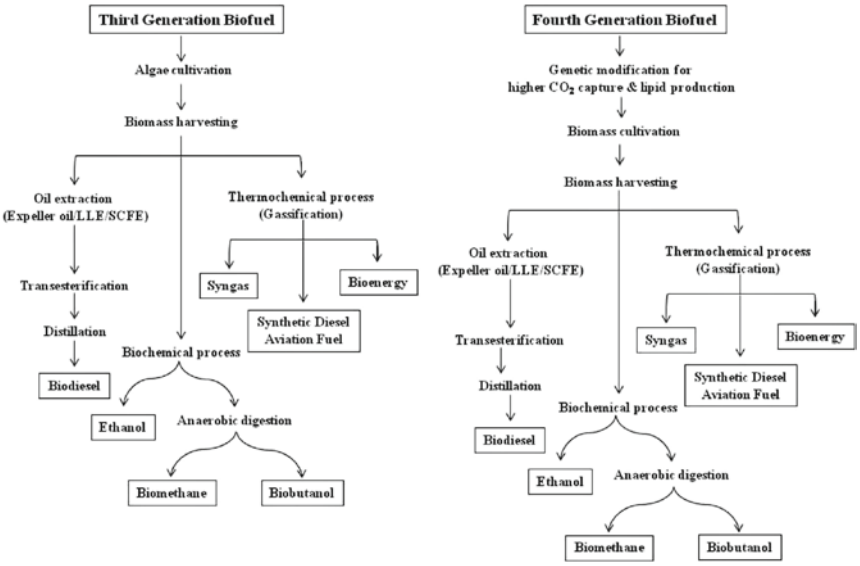


Figure 1.4. Third and fourth generation biofuels. Reproduced from [19] with permission.

Biofuels produced from algae are termed third generation biofuels (Figure 1.4). In the case metabolic engineering is applied to the algae to improve productivity, the biofuels derived thereof are known as fourth generation biofuels [19].

However, commercial units using algae as biomass input have not been realised yet and significant technological advances are required to reduce production cost [21].

4. Use of plant oils for biofuel production

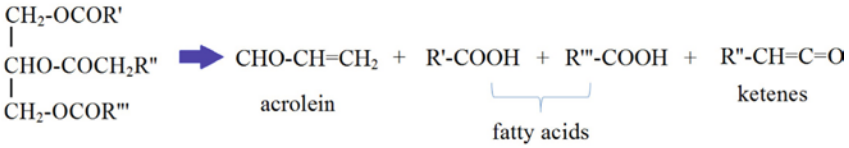
The direct use of plant oils in combustion engines or blending with diesel fuel has been investigated since 1900 onwards [22]. However, the direct use of plant oils has some major drawbacks which are related to the intrinsic properties of the plant oils. Examples are a high viscosity, low volatility, in some cases a relatively high free fatty acid content (FFA), and the presence of carbon deposits [23,24]. Upgrading of the plant oils has been proposed to improve the product properties. Examples include pyrolysis, micro-emulsification, catalytic hydrotreatment and transesterification. These methods will be discussed in the next sections. The main focus will be on biodiesel production by transesterification, as this is the technology investigated in detail in this thesis.

4.1. Pyrolysis

Pyrolysis is an example of a thermochemical conversion technology and involves heating the biomass (here plant oil) to elevated temperatures (400-600°C) in the absence of air, possibly in combination with catalysts [25]. Pyrolysis can be applied to plant oils, animal fats, natural fatty acids or methyl esters of fatty acids [22,26]. Pyrolysis leads to biofuels with higher cetane numbers, lower viscosities and lower water contents than the original plant oil. However, the ash content and carbon residue can be high and in some cases the pour points are also not on-spec [23].

Thermal pyrolysis is mostly conducted at temperatures between 300 – 500°C and involves conversion of the triglycerides to acrolein, fatty acids, and ketenes. These are typically not stable at reaction conditions and undergo subsequent reactions to final products (Figure 1.5) [26]. Catalytic pyrolysis may also be applied and is typically involves the use of acidic zeolites. The triglycerides are cracked to hydrocarbons and fatty acids on the surface of the catalysts. These products are then converted into light alkenes and alkanes, water, carbon dioxide, and carbon monoxide. Highly aromatic, gasoline-type final products have been obtained when using catalytic pyrolysis technology [26].

(1) Decomposition of triglyceride



(2) Decomposition of fatty acids

$$RCOOH \rightarrow CO_2 + RH$$
$$2RCOOH \rightarrow CO_2 + H_2O + RCOR$$

(3) Decomposition of ketenes and acrolein

$$2R''CH=C=O \rightarrow 2CO + R''HC=CHR''$$
$$CH_2=CHCHO \rightarrow CO + C_2H_4$$
$$RCOCH_2R \rightarrow R–R + CH_2CO$$
$$2RCOCH_2R \rightarrow 2R_2 + CO + C_2H_4$$

Figure 1.5. Thermal decomposition of triglycerides, reproduced from [26] with permission

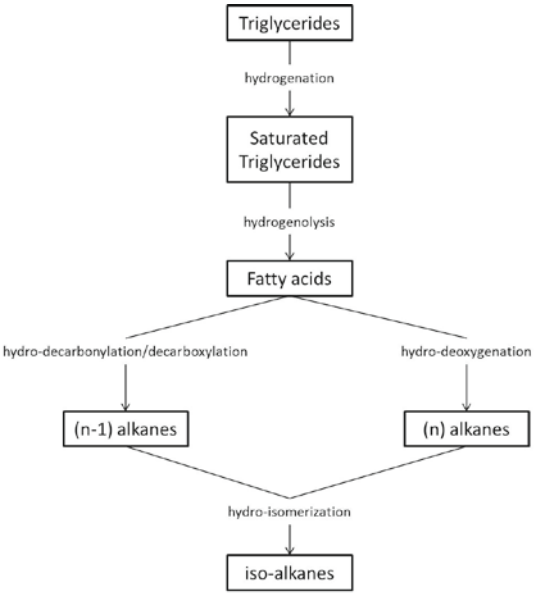


Figure 1.6. Main reaction pathway of plant oil catalytic hydrotreatment according to [29].

4.2. Microemulsification

Microemulsification is a method to reduce the viscosity of plant oils. It involves mixing of low molecular weight alcohols with a biodiesel-diesel mixture using

surfactants for stabilization. The resulting micelles are small (10 – 100 nm) resulting in isotropic micro-emulsified fuels [27]. Fuels obtained by microemulsification do not require modification of the diesel engine [28] though a major drawback is the use of expensive surfactants.

4.3. Catalytic hydrotreatment

Plant oils can be transformed into liquid alkanes using hydrogen at high pressures and moderate temperatures in the presence of supported metal catalysts, a process commonly referred to as hydrotreating [29]. The paraffinic products are suitable to be used as diesel and jet fuel and termed Hydrotreated Vegetable Oils (HVOs) [29,30]. The main reaction pathways are depicted in Figure 1.6. [29].

Catalytic hydrotreatment has been applied to various plant oils, such as rape seed oil [31], palm oil [31,32], Jatropha oil [31,33], sunflower oil [34], and cotton seed oil [35]. Pilot plant scale hydroprocessing has been done for palm oil and resulted in a highly paraffinic renewable diesel with an excellent cetane index [32]. Application of HVOs in compression ignition (CI) engines was shown to lead to a reduction in NOx, PM, HC and CO emissions [30]. HVO production has been commercialised by Neste Corporation (Finland) [17].

4.4. Biodiesel

The most important biofuel derived from plant oils is biodiesel. A biodiesel is defined as "a mixture of mono-alkyl esters of a long chain fatty acids derived from vegetable oils or animal fats which conform to ASTM D6751 specifications for use in diesel engines". Biodiesel can be used in common diesel engines without major modification, is biodegradable, produces less GHG emissions than diesel, has a low toxicity, and comparable performance compared to diesel fuel [25,36–40]. It was reported that the use of biodiesel leads to improved combustion performance, resulting in lower hydrocarbon, carbon monoxide and smoke emissions compared to fossil diesel [37]. On the other hand, NO_x and CO_2 emissions are higher (12 and 14%, respectively), even though the total net CO_2 emission to the environment is small when considering the complete life cycle. Employment of post treatment exhaust gas cleaning was used to reduce the NO_x emissions [25,37].

Biodiesel is produced by transesterification, a reaction of a triglyceride with methanol (though other alcohols may also be used) in the presence of a catalyst to produce esters with glycerol as the by-product (Figure 1.7). Methyl esters

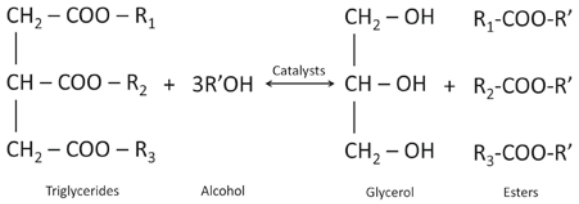


Figure 1.7. Transesterification of triglycerides

are commonly produced, though ethanol has some advantages (higher reaction rates than methanol and easily obtained from renewable resources) [41].

The transesterification reaction can be catalysed by acids, bases, or enzymes; either in homogenous or heterogeneous form. Inorganic bases are commonly used in biodiesel production units due to their low cost and high reactivity at low temperatures. Well known examples are sodium and potassium hydroxide [42]. Recently, enzymes have been introduced for biodiesel synthesis and shown to

Table 1.2. Biodiesel feedstocks [25]

Country	Feedstock
USA	Soybeans/waste oil/peanut
Canada	Rapeseed/animal fat/soybeans/yellow grease and tallow/mustard/flax
Mexico	Animal fat/waste oil
Germany	Rapeseed
Italy	Rapeseed/sunflower
France	Rapeseed/sunflower
Spain	Linseed oil/sunflower
Greece	Cottonseed
UK	Rapeseed/waste cooking oil
Sweden	Rapeseed
Ireland	Frying oil/animal fats
India	Jatropha/*Pongamia pinnata* (karanja)/soybean/rapeseed/sunflower/peanut
Malaysia	Palm oil
Indonesia	Palm oil/coconut
Singapore	Palm oil
Philippines	Coconut/Jatropha
Thailand	Palm/Jatropha/coconut
China	Jatropha/waste cooking oil/rapeseed
Brazil	Soybeans/palm oil/castor/cotton oil
Argentina	Soybeans
Japan	Waste cooking oil
New Zealand	Waste cooking oil/tallow

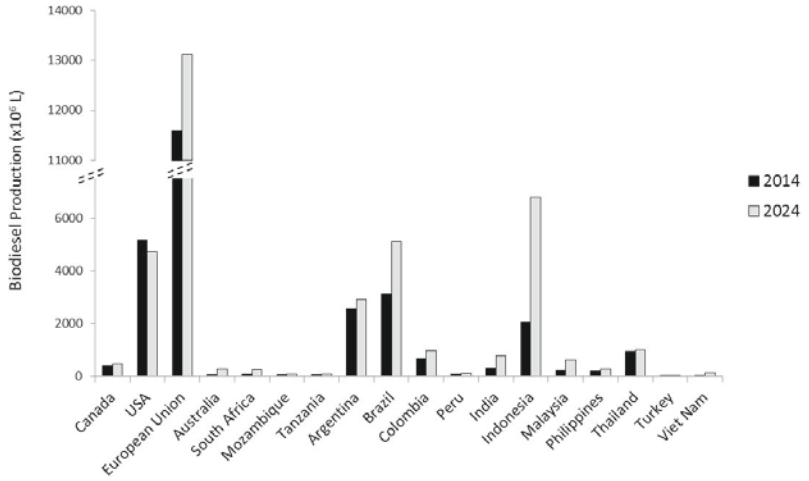


Figure 1.8. Global biodiesel production in 2014 and estimated values for 2024 [44].

have some major advantages compared to acids and bases. These include a better compatibility with variations in feedstock quality, good possibilities for reuse, tolerance for feeds with high FFA contents and the possibility to reduce the number of unit operations due to a less complicated product work-up (no neutralisation step required) [42,43]. On the other hand, low reaction rates, high cost, and loss of activity after repeated usage are disadvantages of enzymes [43].

The oil feeds can be divided into four categories: (1) edible vegetable oils (soybeans, palm oil, sunflower, safflower, rapeseed, coconut, and peanut), (2) non-edible vegetable oils (Jatropha, karanja, sea mango, algae, and halophytes), (3) waste or recycled oils, and (4) animal fats (tallow, yellow grease, chicken fat, and fish oil by-products). Edible vegetable oils are typically used as feedstock (Table 1.2), and considered as first generation biodiesel [25]. However, the use of edible oils for biodiesel production is in competition with the food sector. As such, biodiesel from non-edible oils and waste oils, considered as second generation biodiesel, is gaining high attention at the moment [23].

Global biodiesel production in 2014 reached 27.9 bln L and is estimated to increase with 2.1% per year to 38.6 bln L in 2024 (Figure 1.8). Most of the biodiesel is produced in EU countries [44]. The highest increase in biodiesel production is estimated for Indonesia, followed by EU countries and Brazil. Although non-EU countries have potential feedstock for second generation biodiesel (Table 1.2), the production technology has not been implemented on large scale [25].

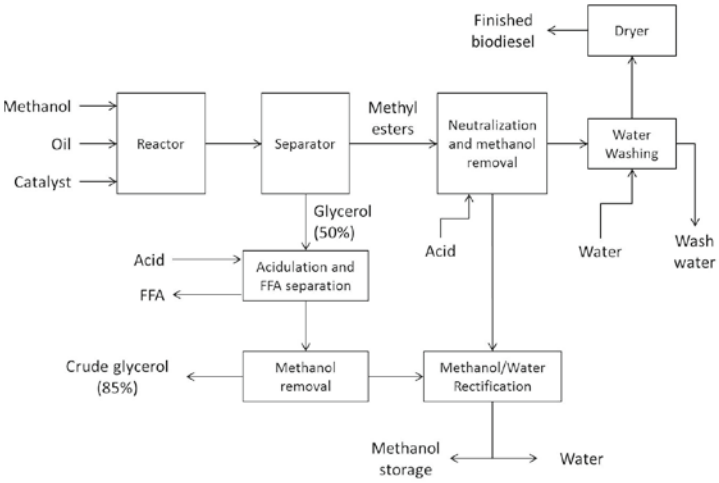


Figure 1.9. Overview of a typical alkali catalysed biodiesel process, redrawn from [45].

4.5. Biodiesel technology

Batch reactors are commonly used in the biodiesel industry. Most of the larger plants, with production capacities more than 4 million litres per year, use continuous stirred-tank reactors (CSTR), or plug flow reactors [45]. A schematic representation of a typical biodiesel production unit using a base catalyst is presented in Figure 1.9.

Several challenges have been identified regarding biodiesel production. For instance, mass transfer limitations between the immiscible oil and alcohol phase are known to reduce the overall reaction rates. In addition transesterification is an equilibrium reaction which limits the conversion [46]. In order to overcome these problems, long reaction times, high alcohol to oil ratios and high catalyst concentrations are used. However, this results in high operating costs and energy consumption, for instance to purify/neutralise the biodiesel and to recover the excess of alcohol and catalyst. Significant amounts of waste water are also produced in the downstream processing units [46].

4.6. New developments in biodiesel technology

Process intensification in both the reactor and work-up section has been proposed to reduce the manufacturing costs of biodiesel. Examples of intensified technologies are the use of either novel reactor configurations (static mixers, micro-channel

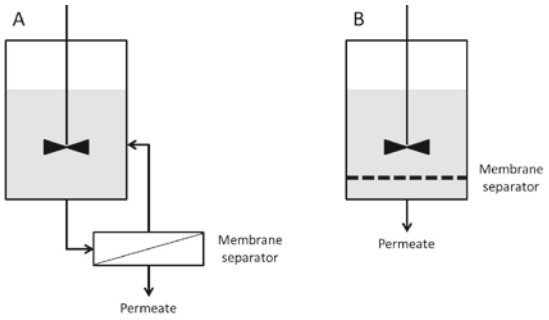


Figure 1.10. Basic layout of a conventional (A) and integrated (B) membrane reactor [47].

reactors, oscillatory flow reactors, cavitational reactors, rotating/spinning tube reactors, microwave reactors) or combination of reaction and separation processes (membrane reactors, reactive distillation, centrifugal contactors) [46]. Process intensified devices combining reaction and separation are discussed in detail in the following sections.

4.6.1. Membrane reactors

Membrane processes involve the use of selective membranes for separation by differences in mass transfer rates of components in the membrane [47]. The idea of combining membrane separation processes and reaction in a single device has attracted substantial interest since the early 90's as it may lead to substantial reductions in capital and processing costs. When combined with a catalytic reaction, a higher selectivity and yield can be obtained by shifting chemical equilibrium compositions due to the continuous extraction of products [48]. Two basic layouts of membrane reactors are depicted in Figure 1.10.

Membrane reactors are considered attractive for use in the biodiesel industry. It was reported that the use of these reactors allow for separation of the glycerol and excess alcohol [49,50], along with unreacted oil [51] from the fatty acid alkyl ester (FAAE) product. The recovered alcohol can be recycled into the reaction leading to lower alcohol usage [52].

4.6.2. Reactive distillation

Reactive distillation, or catalytic distillation, is a combination of reaction and distillation in a single distillation column. The catalytic process can involve both homogenous and heterogeneous catalysts [53]. The technology has attracted

high attention because conversion limitations due to equilibrium constraints can be overcome by continuous *in situ* removal of the product [46].

He *et al.* introduced a novel continuous reactor using the reactive distillation principle to produce biodiesel from canola oil and methanol. Several advantages were reported compared to conventional operation, *viz.* a reduction of the methanol usage with 66% and considerably higher reaction times [54]. The reactor concept was optimized and biodiesel yields up to 98.9% were reported [55]. The successful application of reactive distillation to produce biodiesel from other plant oils such as soybean oil [56] and derivatives (oleic acid) has also been reported [57].

Poddar *et al.* performed a techno-economic analysis of biodiesel production using reactive distillation and found that production costs and capital expenditure using heterogeneous catalysts were lower than when using homogenous catalysts. This study also showed that the manufacturing cost of the product was slightly higher than the current biodiesel price [58].

4.6.3. Centrifugal contactor separators

A centrifugal contactor separator (CCCS) is a device that combines mixing and separation for liquid-liquid extractions in a continuous mode. It consists of a cylindrical rotor in a static outer house. The rotor is hollow and acts as a centrifuge. The outer annular zone may be considered as the mixing zone, whereas the centrifuge is used for liquid-liquid separation (Figure 1.11) [59,60].

A number of studies have been performed to use the device for transesterification reactions of plant oils. For instance, Kraai *et al.* [61] explored the effects

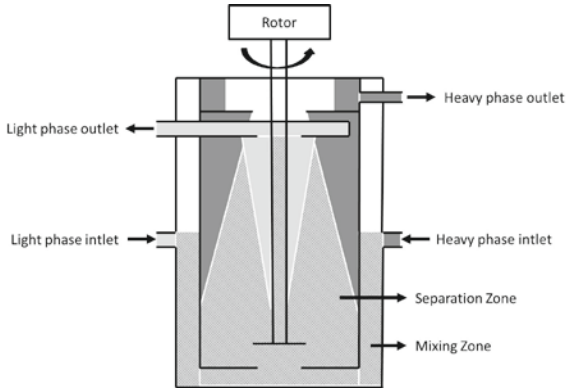


Figure 1.11. Schematic representation of a centrifugal contactor separator, redrawn from [61].

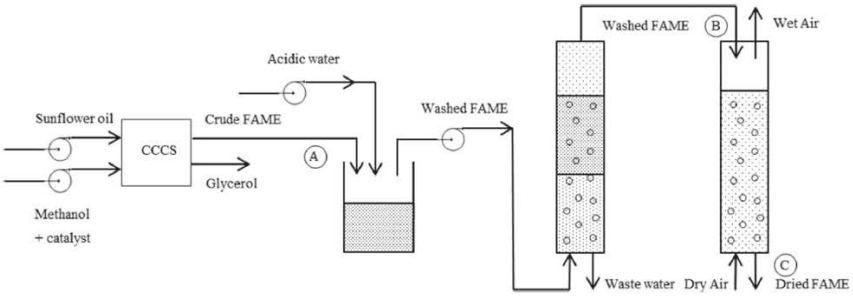


Figure 1.12. Schematic representation of a continuous biodiesel bench scale unit using a cascade of a CCCS and a conventional work-up section. Reproduced from [64] with permission.

of process parameters (catalyst loading, temperature, rotational frequency, and flow rates) for biodiesel production from sunflower oil and methanol using a base catalyst. At optimized conditions, a reproducible 96% biodiesel yield was obtained. In addition, it was shown that the volumetric productivity was slightly higher than for conventional batch processes (61 $kg_{FAME}m^{-3}{}_{liquid}min^{-1}$). Abduh *et al.* [62] studied the transesterification of Jatropha oil with ethanol in the CCCS device and 98% product yield at a productivity of 112 $kg_{FAEE}m^{-3}{}_{liquid}min^{-1}$ were obtained at optimized conditions.

The CCCS device can be easily coupled to other reactors in a cascade configuration to allow for high product yields and productivities in combination with *in situ* phase separation. For instance, a cascade of two CCCS reactors was used in an optimisation study for biodiesel synthesis from sunflower oil and methanol by Abduh *et al.* [63]. The first CCCS acted as the reactor and product separator, while the second CCCS was used to purify/work-up the crude biodiesel. The FAME yield at optimized conditions was 94%. In a subsequent study, Abduh *et al.* [64] combined the CCCS for reaction and product separation followed by a conventional work-up section (washing unit and drying column, Figure 1.12) for sunflower oil methanolysis. The FAME yield was 98% which is higher than reported for their previous study using a cascade of two CCCS devices [63]. Refined products from both process options met the ASTM specifications.

Centrifugal contactor reactors are compact in size, robust, and flexible in operation. These, in combination with high volumetric productivities, make them very attractive devices to be used in mobile biodiesel production units [61–64]. The units can be deployed in rural areas, especially in developing countries, to produce biodiesel to fulfil local energy demands.

5. Enzymatic biodiesel synthesis

Inorganic acids and bases are typically used as catalysts in the biodiesel industry. Among them, alkaline catalysts are preferred because they are cheap and lead to high reaction rates. For instance, the alkaline-catalysed transesterification reaction rates are nearly 4000 times faster than when using inorganic acids [65]. However, several disadvantages have been identified when using alkaline catalysts. Homogenous alkaline catalysts are known to lead to saponification of FFAs, generating emulsions which are difficult to break and which lead to serious issues in downstream processing. Heterogeneous alkaline catalysts still suffer from saponification, are prone to catalyst leaching, and are prepared using tedious preparation routes [66].

Enzymes can also be used to convert plant oils into biodiesel [66]. The use of enzymes can overcome some of the disadvantages of conventional chemical catalysts. Enzymatic conversions give a higher product purity, require less energy input and enable the use of a wider range of feedstocks including FFAs [66,67]. On the other hand, enzymes are sensitive to alcohols and (partial) deactivation of lipases in methanol and ethanol results in lower product yields [66,67]. In general, enzymes are more expensive than base catalysts [66,67].

5.1. Properties of lipases

Lipases (EC 3.1.1.3) are carboxylesterases that catalyse the hydrolysis of, among others, triglycerides. They are produced by all living organisms, the molecular sizes of lipases range from 19 to 60 kDa and they show a characteristic folding pattern known as the α/β-hydrolase fold. Lipases are active in a pH range between 7.5 and 9, and a temperature range of 35 - 50°C for mesophilic lipases and between 60 – 80°C for thermophilic lipases [67,68].

An overview of typical reactions catalysed by lipases is given in Figure 1.13 [69]. Lipases not only catalyse transesterifications, but also effectively esterify fatty acids. This is a major advantage over conventional base catalysts as lipases tolerate substantially higher amounts of FFAs in the feed [69].

Lipase catalysed reactions typically occur at the lipid-water interface. The hydrophobic active site of the enzyme is covered by a peptide lid which will be open when exposed to hydrophobic substrates [68].

Plant lipases have been obtained from a wide range of sources, such as papaya latex, rapeseed, oat, and castor seeds; animal lipases can be obtained from the pancreas of sheep, hogs, and pigs [70]. Many microorganisms produce lipases (Table 1.3). The synthesis of lipases from bacteria is well established,

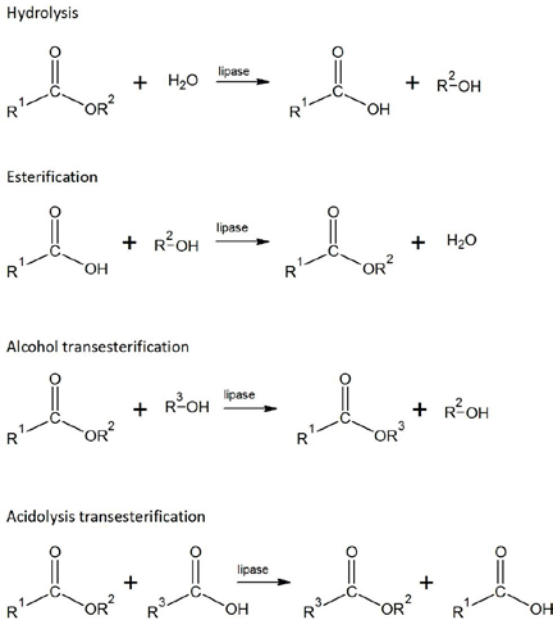


Figure 1.13. Reactions catalysed by lipases

Table 1.3. Lipase-producing microorganisms [67]

Bacteria		Filamentous fungi	Yeast
Achromobacter lipolyticum	*Proteus vulgaris*	*Alternaria brassicicola*	*Candida deformans*
Acinetobacter radioresistens	*Pseudomonas aeruginosa*	*Aspergillus fumigates*	*C. parapsilosis*
A. calcoaceticus	*P. cepacia*	*A. japonicas*	*C. rugosa*
A. pseudoalcaligenes	*P. fragi*	*A. nidulans*	*C. quercitrusa*
Aeromonas hydrophila	*P.mendocina*	*Candida antarctica*	*Pichia burtonii*
Archaeglobus fulgidus	*P. nitroreducens var. thermotolerans*	*Mucor miehei*	*P. sivicola*
Bacillus acidocaldarius		*Penicilliumcyclopium*	*P. xylosa*
B. megaterium	*Pseudomonas sp.*	*Rhizomucor miehei*	*Saccharomyces lipolytica*
B. pumilus	*Psychrobacter immobilis*	*Rhizopus arrhizus*	*Geotrichum candidum*
Bacillus sp.	*Serratia marcescens*	*R. chinensis*	*Yarrowia lipolytica NRRL YB-423*
B. stearothermophilus	*Staphylococcus aureus*	*R. microsporous*	
B. subtilis	*S. canosus*	*R. niveus*	
B. thermocatenulatus	*S. epidermidis*	*R. nodosus*	
B. thermoleovorans	*S. haemolyticus*	*R. oryzae*	
Burkholderia glumae	*S. hyicus*	*Streptomyces cinnamomeus S. exfoliates*	
Chromobacterium viscosum	*S. warneri*		
Enterococcus faecalis	*S. xylosus*	*S. fradiae*	
Micrococcus freudenreichii	*Sulfolobus acidocaldarius*	*Streptomyces sp.*	
Moraxella sp.	*Vibrio chloreae*	*Aspergillus niger*	
Mycobacterium chelonae	*Pseudomonas alcaligens*	*Thermomyces lanuginous*	
Pasteurella multocida	*P. putida*	*Fusarium heterosporum*	
Propionibacterium acnes	*Chromobacterium visosum*	*Humicola lanuginose*	
P. avidium	*Statphylococcus stolonifera*	*Oospora lactis*	
P. granulosum			

though fungal lipases are easier to produce and handle. Among the fungi derived lipases, *Candida* and *Rhizomucor* have been commercialized [67] such as Lipozyme® CALB and Palatase® produced by Novozymes Corp., Denmark.

5.2. Biodiesel synthesis using soluble lipases

Batch reactors are considered as the most suitable reactor for biodiesel production using soluble lipases (also known as liquid enzyme formulations), however some authors described the use of continuous reactors. In the following, the use of both reactor configurations will be discussed.

5.2.1. Biodiesel synthesis using soluble lipases in batch reactors

Biodiesel synthesis using soluble lipases in batch reactors has been reported, though recovery and recycle of the enzyme is not covered in great detail [71,72]. Scale-up studies have been reported by several research groups using a Callera™ Trans L lipase (Novozyme) [73,74] and Eversa Transform lipase (Novozyme) liquid formulation [75]. Price *et al.* [73] performed a modelling study on biodiesel production using a liquid lipase formulation in a batch reactor. A simulated biodiesel yield of 90.8 wt% was reported at optimum parameters for the model. Another study [74] involved modelling of various feeding strategies in fed-batch biodiesel production units using liquid lipase formulations and reported a 37% increase in reactor productivity compared to a conventional batch reactor. Nielsen *et al.* [75] investigated a novel process configuration involving a combination of enzymatic biodiesel production in batch using a liquid lipase formulation followed by a saponification step to reduce the FFA content to values less than 0.25%. A biodiesel yield of up to 97% was reported.

5.2.2. Biodiesel synthesis using soluble lipases in continuous reactors

Enzymatic biodiesel production in continuous reactors is considered more economical than in batch reactors [76]. However, most studies in continuous set-ups focussed on immobilized lipases and literature reports on the use of soluble lipase formulations for biodiesel production in continuous reactors are scarce. Price *et al.* [77] studied enzymatic biodiesel production in a continuous mode using a liquid Callera™ Trans L lipase formulation (Novozyme) [74]. Based on the results, it was predicted that a cascade of 5 continuous stirred

tank reactors (CSTRs) with a total residence time of 30 hours was required to obtain a biodiesel yield of 95.6 wt%. A pilot plant study on the continuous biodiesel production using NS-40116 (a liquid formulation of a modified *Thermomyces lanuginosus* lipase) was reported involving a cascade of 4 CSTRs (total volume 16 m^3) [78]. The plant output was optimized to reach up to 852 ton product per year.

5.3. Biodiesel production using immobilized lipases

One way to overcome the high costs of lipases is to immobilise them. Immobilization of enzymes, defined as the physical confinement of the enzyme, is often performed using an organic or inorganic matrix [79]. Several carriers have been reported for commercial immobilized lipases, including Lewatit VP OC 1600 (Novozyme® 435), Duolite A568 (Lipozyme® RM IM), silica granules (Lipozyme® TL IM), and diatomaceous earth (Lipase PS Amano IM) [80]. Advantages of immobilized lipases are the easy of recovery and reuse, higher adaptability for continuous operation, less effluent problems and a higher thermal stability and lower sensitivity to pH [80]. The use of immobilised enzymes has been studied in four reactor configurations: batch reactors, continuously stirred tank reactors, fixed bed reactors, and fluidized bed reactors [76]. The literature regarding the first two options will be discussed in detail in the following sections, as these are the most relevant for the research described in this thesis.

5.3.1. Use of immobilised enzymes in batch reactors

Small (lab-)scale biodiesel synthesis with immobilised lipases is typically carried out in a batch reactor equipped with a mechanically stirrer, usually a propeller or Rushton turbine. The catalyst particles are typically dispersed in the substrate solution [76]. The batch reactor offers several advantages such as good catalyst and substrate dispersion, high mixing intensities, thereby reducing mass transfer limitations and simplicity [81]. An issue is a reduction in efficiency due to disruption of the enzyme carrier due to the high shear induced by the stirrer [81].

Biodiesel synthesis using immobilized enzymes in batch reactors has been studied extensively on small scale. Relevant studies using immobilized enzymes in batch reactors are summarized in Table 1.4. Most involve exploratory catalyst screening studies, optimisation [82–86] and kinetic studies [87–90]. High biodiesel yields are possible, not only with methanol but also with higher alcohols.

Table 1.4. Overview of biodiesel synthesis by immobilized lipases using batch reactors.

Lipases and supports	Substrate	Alcohol	Highest Yield	Remarks	Ref.
Candida sp. 99-125 on cotton membrane	Vegetable oil	Methanol	96%	Methanol to oil ratio = 3:1 Temp. 40°C, 170 rpm (reciprocation) High water content (5 – 20%)	[82]
C. antarctica (CALB), *T. lanuginosus* (TLL), and *R. miehei* (RML) on SBA epoxy	Canola oil	Methanol	CALB-SBA: 59% TTL-SBA: 99% RML-SBA: 95%	Methanol to oil ratio = 3:1 50°C, 250 rpm magnetic stirring	[83]
T. lanuginosus on silica gel	Palm oil	Methanol	99%	Methanol to oil ratio = 1:3.7 150 rpm (reciprocation)	[84]
P. fluorescen on porous kaolinite	Triolein	1- propanol	>99%	Propanol to oil ratio = 3:1 50°C, continuous stirring	[85]
Candida sp. 99-125 on cotton membrane	Glycerol trioleate	Methanol	80.6%	Methanol to oil ratio = 3:1 Temp. 40°C, 180 rpm (reciprocation) Water content 20%	[86]
Rhizomucor miehei on macroporous anionic exchange resin	Palm oil	Oleyl alcohol	1.5 L: 95.8% 50 L: 97.2%	Alcohol to oil ratio = 3:1 Temp. 50°C 250 rpm, propeller (1.5 L) 106 rpm, propeller (50 L) Enzyme activity after 15 cycles: 79%	[91]

Keng *et al.* [91] successfully scaled up the process from 1.5 L to 50 L using a constant impeller tip speed approach. The biodiesel yield was 97.2% after 5 h reaction time. Large scale industrial applications of batch reactors involving immobilised enzymes for biodiesel synthesis, though, was reported to be not economically feasible due to low volumetric production rates as a result of unproductive time required to unload, clean, and reload the reactor. Moreover, enzyme activity was shown to decrease after reuse of the catalyst, presumably due to shear stress, resulting in the need for longer reaction times to achieve the required conversions in subsequent steps. It is considered to be difficult to find a suitable compromise between production capacity and catalyst cost [92].

5.3.2. The use of immobilized lipases in continuous stirred tank reactors

In a continuous stirred tank reactor (CSTR), the immobilised enzymes are dispersed in the substrates/products. Multiple tanks in series, either with a separation unit at the end or with intermediate separation units are commonly used [71]. Fonseca *et al.* [93] performed simulations on biodiesel production

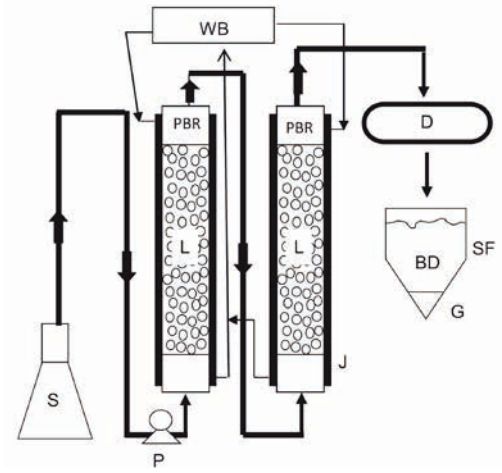


Figure 1.14. Continuous biodiesel production in a CSTR-PBR cascade system. MeOH: methanol, P: pump, J: water circulating jacket, L: immobilized lipase, WB: water bath, CSTR: continuous stirred tank reactor, PBR: packed bed reactor, D: distillation unit, SF: separating funnel, BD: biodiesel, G: glycerol layer. Reproduced from [95] with permission.

using immobilised enzymes from soybean and palm oil in batch and CSTR reactors. They found that a cascade of 3 or more CSTRs is needed to achieve the same productivity as for a single batch reactor operated at the same reaction time. Moreover, they showed that a single CSTR requires a 373 times higher residence time, compared a batch reactor to achieve 95% productivity. When using two CSTRs in series, the residence time could be reduced with a factor of 4.

A major concern when using CISTRs for enzymatic biodiesel synthesis is enzyme deactivation which requires that at least part of the catalyst is replaced with fresh catalyst during the reaction. Chen and Wu [94] showed that treatment of spent *Candida antartica* lipase with t-butanol is an interesting approach to reactivate the enzyme. The authors demonstrated the concept for biodiesel synthesis in a CSTR using soybean oil and methanol as the feed and succeeded to maintain a constant conversion level of 70% for 70 days.

A cascade consisting of a CSTR followed by two fixed bed reactors has been explored for biodiesel synthesis. Six different oils were used and an immobilized Streapsin lipase was used as the catalyst (Figure 1.14). The CSTR was the first reactor in the cascade, and acted mainly as a mixer to allow for good dispersion of the two immiscible substrates. An average 72% conversion was obtained after 40 h with a biodiesel productivity of 137.2 g L^{-1} h^{-1}. This is higher than reported for biodiesel synthesis in conventional continuous set ups using immobilized enzymes [95].

Table 1.5. Protein content of various oil plant seed cakes

Source plant	Protein content (wt%)	References
Canola	33.9	[96]
Coconut	25.2	[96]
Cotton	40.3	[96]
Ground nut	49.5	[96]
Mustard	38.5	[96]
Olive	6.3	[96]
Palm	18.6	[96]
Sesame	35.6	[96]
Soybean	47.5	[96]
Sunflower	34.1	[96]
Rubber	19 - 23	[99]
Jatropha curcas	18.3 - 65.6	[100]

6. Oil plant seed cake valorisation

After the extraction of the plant oil from plant seeds, a protein rich organic residue remains. The residue, also known as seed cake, is rich in proteins (Table 1.5) and has been considered for many applications such as animal feed, fertilizer, protein concentrate, and biogas production [96–98]. The press cake has been used to produce various microbial enzymes such as proteases, glucoamylases, and lipases [96]. In this case, the press cake provides the relevant substrates for the microbes to grow [96].

Most of studies on seed cake utilisation were focused on solid-state fermentation (SSF) [101]. Typically SSF involves cultivation of microorganisms using a dry or moist solid substrate such as wheat straw as carbon and energy source [102]. Major issues on larger scale application have been found despite attractiveness of SSF method. It is difficult to maintain a constant micro-environment, for instance the temperature can increase rapidly as a result of the microbial growth. This is problematic, especially when temperature sensitive substances are produced such as enzymes. The use of submerged fermentation (SmF) eliminates some of these issues and is considered a preferred method [103].

7. About this thesis

The research described in this thesis was part of a project titled "Breakthroughs in biofuels: Mobile technology for biodiesel production from Indonesian resources" funded by NWO/WOTRO. In this project, the biorefinery concept was

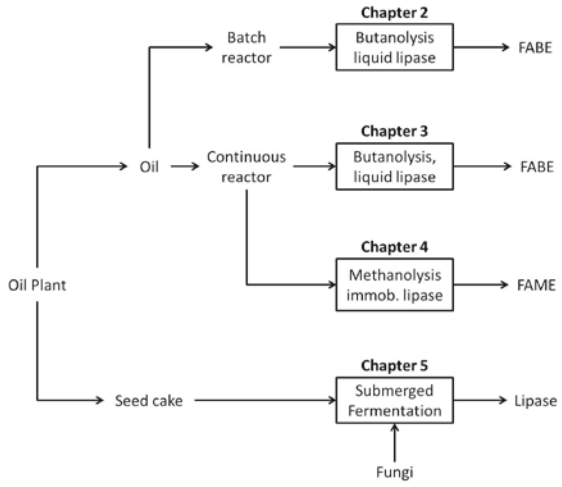


Figure 1.15. Concept outline of this thesis

explored for the valorisation of rubber seeds in Indonesia, with an emphasis on utilisation of the oil and seed cake. The first objective of the study described in this thesis was to provide the proof of principle for continuous biodiesel synthesis using enzymes, both homogeneous as well as heterogeneous, in highly intensified process equipment and in particular in CCCS devices. The second objective was to define the potential of plant oil seed cakes, the residue after seed processing to oil, for the production of enzymes, and in particular lipases for the subsequent biodiesel synthesis. Rubber seed oil was not available in sufficient quantities for continuous operation in the first stage of the project and as such most experimental studies were carried out using plant oils like sunflower and Jatropha oil. An overview of the thesis chapters is given in Figure 1.15.

In Chapter 2, experimental and modelling studies aimed to develop a kinetic model for the reaction of a plant oil (sunflower oil) with 1-butanol catalysed by a liquid lipase formulation (*Rhizomucor miehei*) in an aqueous (enzyme/water)-organic system (oil/hexane) are provided. The reactions were performed in a stirred batch reactor. Enzyme concentration, stirring speed, and 1-butanol to oil ratio were systematically varied. A kinetic model was developed based on concentration-time profiles for the reaction. The experimental data were successfully modelled using a Ping Pong Bi Bi mechanism with non-competitive inhibition by 1-butanol and a term for irreversible enzyme deactivation during reaction.

In Chapter 3, experimental and modelling studies on the reaction of sunflower oil with 1-butanol in a biphasic water/organic system using a liquid enzyme formulation (*Rhizumucor miehei)* are reported in various continuous reactor configurations. The reaction was first optimized in a continuous stirred tank reactor (CSTR) focusing on enzyme concentration and residence times as the

main variables. A reactor model was developed using the kinetic expression as described in Chapter 2. In a second stage of experimentation, a cascade consisting of a CSTR followed by a continuous centrifugal contactor separator (CCCS) was used. Product yields and productivities were determined in the cascade and recyclability of the lipase was explored.

In Chapter 4, experimental and modelling studies on the enzymatic methanolysis of sunflower oil using an immobilized lipase (TransZyme A) is reported. In the initial stage, experiments were carried out in a batch configuration to optimize enzyme and buffer concentrations. The results were modelled using Michaelis-Menten kinetics. Subsequent experiments were performed in a CSTR and CCCS and a reactor model was developed using the kinetic expression obtained in batch as input. In addition, experimental studies were performed in a cascade consisting of a CSTR and CCCS and biodiesel yield, enzyme stability, and separation performance were evaluated.

In Chapter 5, experimental studies on the use of the Jatropha seed-cake as substrate for fungal lipase production will be discussed. The Indonesian indigenous fungal strain, *Aspergillus niger* 65I6, and a well-known lipase producing fungus, *Rhizomucor miehei* CBS 360.62 [7], were selected as fungi of choice. The effects of particularly the pre-treatment of the seed-cake with base and the type of carbon sources in the growth medium were evaluated in detail.

References

[1] International Energy Agency, Key World Energy Statistics 2015, Paris, 2015.

[2] BP, Sustainability Report 2015, London, 2015.

[3] H. Embree, Oxygenated aromatic compounds from renewable resources: motivation, opportunities, and adsorptive separations, Chem. Eng. J. 84 (2001) 133–147.

[4] F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, Energy Convers. Manag. 51 (2010) 1412–1421.

[5] BP, Statistical Review of World Energy 2015, London, 2015.

[6] IPPC, Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge (UK) & New York (USA), 2007.

[7] REN21, Renewables 2015 Global Status Report, REN21 Secretariat, Paris, 2015.

[8] H.R. Ghatak, Biorefineries from the perspective of sustainability: Feedstocks, products, and processes, Renew. Sustain. Energy Rev. 15 (2011) 4042–4052.

[9] K. Mollersten, J. Yan, J.R. Moreira, Potential market niches for biomass energy with CO2 capture and storage - Opportunities for energy supply with negative CO2 emissions, Biomass and Bioenergy. 25 (2003) 273–285.

[10] K. Mollersten, L. Gao, J. Yan, CO_2 capture in pulp and paper mills: CO_2 balances and preliminary cost assessment, Mitig. Adapt. Strateg. Glob. Chang. 11 (2006) 1129–1150.

[11] U.S. Department of Energy Office of Science, Genomics: GTL Roadmap: Systems Biology for Energy and Environment, 2005.

[12] B. Vanholme, T. Desmet, F. Ronsse, K. Rabaey, F. Van Breusegem, M. De Mey, et al., Towards a carbon-negative sustainable bio-based economy., Front. Plant Sci. 4 (2013) 174.

[13] European Commission, Bio-based economy in Europe: state of play and future potential - Part 2 - Summary of the position papers received in response to the European Commission's Public on-line consultation, Publications Office of the European Union, Luxembourg, 2011.

[14] O. Schmid, S. Padel, L. Levidow, The bio-economy concept and knowledge base in a public goods and farmer perspective, Bio-Based Appl. Econ. 1 (2012) 47–63.

[15] E. de Jong, G. Jungmeier, Biorefinery Concepts in Comparison to Petrochemical Refineries, in: A. Pandey, R. Hofer, M. Taherzadeh, K.M. Nampoothiri, C. Larroche (Eds.), Ind. Biorefineries White Biotechnol., Elsevier, Amsterdam, 2015: pp. 3–33.

[16] S. Liu, Woody biomass: Niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis, Biotechnol. Adv. 28 (2010) 563–582.

[17] U.S. Energy Information Administration, International Energy Outlook 2016, Washington, 2016.

[18] R. Sims, M. Taylor, J. Saddler, W. Mabee, From 1st- to 2nd-generation biofuel technologies: An overview of current industry and RD&D activities, Paris, 2008.

[19] K. Dutta, A. Daverey, J.-G. Lin, Evolution retrospective for alternative fuels: First to fourth generation, Renew. Energy. 69 (2014) 114–122.

[20] R.E.H. Sims, W. Mabee, J.N. Saddler, M. Taylor, An overview of second generation biofuel technologies, Bioresour. Technol. 101 (2010) 1570–1580.

[21] A. Alaswad, M. Dassisti, T. Prescott, A.G. Olabi, Technologies and developments of third generation biofuel production, Renew. Sustain. Energy Rev. 51 (2015) 1446–1460.

[22] A. Abbaszaadeh, B. Ghobadian, M.R. Omidkhah, G. Najafi, Current biodiesel production technologies: A comparative review, Energy Convers. Manag. 63 (2012) 138–148.

[23] M.M.K. Bhuiya, M.G. Rasul, M.M.K. Khan, N. Ashwath, A.K. Azad, Prospects of 2nd generation biodiesel as a sustainable fuel - Part: 1 Selection of feedstocks, oil extraction techniques and conversion technologies, Renew. Sustain. Energy Rev. 55 (2016) 1109–1128.

[24] E.F. Aransiola, T. V. Ojumu, O.O. Oyekola, T.F. Madzimbamuto, D.I.O. Ikhu-Omoregbe, A review of current technology for biodiesel production: State of the art, Biomass and Bioenergy. 61 (2014) 276–297.

[25] A.E. Atabani, A.S. Silitonga, I.A. Badruddin, T.M.I. Mahlia, H.H. Masjuki, S. Mekhilef, A comprehensive review on biodiesel as an alternative energy resource and its characteristics, Renew. Sustain. Energy Rev. 16 (2012) 2070–2093.

[26] J. Xu, J. Jiang, J. Zhao, Thermochemical conversion of triglycerides for production of drop-in liquid fuels, Renew. Sustain. Energy Rev. 58 (2016) 331–340.

[27] L.D. Do, V. Singh, L. Chen, T.C.G. Kibbey, S.R. Gollahalli, D.A. Sabatini, Algae, Canola, or Palm Oils—Diesel Microemulsion Fuels: Phase Behaviors, Viscosity, and Combustion Properties, Int. J. Green Energy. 8 (2011) 748–767.

[28] E.A. Melo-Espinosa, R. Piloto-Rodríguez, L. Goyos-Pérez, R. Sierens, S. Verhelst, Emulsification of animal fats and vegetable oils for their use as a diesel engine fuel: An overview, Renew. Sustain. Energy Rev. 47 (2015) 623–633.

[29] J.C. Serrano-Ruiz, E. V. Ramos-Fernández, A. Sepúlveda-Escribano, A.J. Ragauskas, R. Luque, L. Herrero-Davila, et al., From biodiesel and bioethanol to liquid hydrocarbonfuels: new hydrotreating and advanced microbial technologies, Energy Environ. Sci. 5 (2012) 5638–5652.

[30] S.-Y. No, Application of hydrotreated vegetable oil from triglyceride based biomass to CI engines – A review, Fuel. 115 (2014) 88–96.

[31] R. Arvidsson, S. Persson, M. Fröling, M. Svanström, Life cycle assessment of hydrotreated vegetable oil from rape, oil palm and Jatropha, J. Clean. Prod. 19 (2011) 129–137.

[32] A. Guzman, J.E. Torres, L.P. Prada, M.L. Nuñez, Hydroprocessing of crude palm oil at pilot plant scale, Catal. Today. 156 (2010) 38–43.

[33] Y. Liu, R. Sotelo-Boyás, K. Murata, T. Minowa, K. Sakanishi, Hydrotreatment of Jatropha Oil to Produce Green Diesel over Trifunctional Ni–Mo/SiO2–Al2O3 Catalyst, Chem. Lett. 38 (2009) 552–553.

[34] M. Krár, S. Kovács, D. Kalló, J. Hancsók, Fuel purpose hydrotreating of sunflower oil on CoMo/Al2O3 catalyst, Bioresour. Technol. 101 (2010) 9287–9293.

[35] I. Sebos, A. Matsoukas, V. Apostolopoulos, N. Papayannakos, Catalytic hydroprocessing of cottonseed oil in petroleum diesel mixtures for production of renewable diesel, Fuel. 88 (2009) 145–149.

[36] A.K. Agarwal, Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines, Prog. Energy Combust. Sci. 33 (2007) 233–271.

[37] A. Datta, B.K. Mandal, A comprehensive review of biodiesel as an alternative fuel for compression ignition engine, Renew. Sustain. Energy Rev. 57 (2016) 799–821.

[38] M.M.K. Bhuiya, M.G. Rasul, M.M.K. Khan, N. Ashwath, A.K. Azad, M.A. Hazrat, Prospects of 2nd generation biodiesel as a sustainable fuel - Part 2: Properties, performance and emission characteristics, Renew. Sustain. Energy Rev. 55 (2016) 1129–1146.

[39] A. Shirneshan, N. Amin, Investigation of the effects of biodiesel-diesel fuel blends on the performance and emission characteristics of a diesel engine, J. Teknol. (Sciences Eng. 78 (2016) 169–177.

[40] A. Demirbas, Importance of biodiesel as transportation fuel, Energy Policy. 35 (2007) 4661–4670.

[41] A. Demirbas, Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods, Prog. Energy Combust. Sci. 31 (2005) 466–487.

[42] D.Y.C. Leung, X. Wu, M.K.H. Leung, A review on biodiesel production using catalyzed transesterification, Appl. Energy. 87 (2010) 1083–1095.

[43] L. Fjerbaek, K. V. Christensen, B. Norddahl, A review of the current state of biodiesel production using enzymatic transesterification, Biotechnol. Bioeng. 102 (2009) 1298–1315.

[44] OECD/FAO, Biofuels, Organisation for Economic Cooperation and Development (OECD), 2015.

[45] J. Van Gerpen, G. Knothe, Basics of the Transesterification Reaction, in: G. Knothe, J. Van Gerpen, J. Krahl (Eds.), Biodiesel Handb., 1st ed., AOCS Publishing, Champaign, 2005: p. 328.

[46] Z. Qiu, L. Zhao, L. Weatherley, Process intensification technologies in continuous biodiesel production, Chem. Eng. Process. Process Intensif. 49 (2010) 323–330.

[47] S.H. Shuit, Y.T. Ong, K.T. Lee, B. Subhash, S.H. Tan, Membrane technology as a promising alternative in

biodiesel production: A review, Biotechnol. Adv. 30 (2012) 1364–1380.

[48] J.G. Sanchez Marcano, T.T. Tsotsis, Introduction, in: Catal. Membr. Membr. React., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG, 2002: pp. 1–14.

[49] L. Guerreiro, J.E. Castanheiro, I.M. Fonseca, R.M. Martin-Aranda, A.M. Ramos, J. Vital, Transesterification of soybean oil over sulfonic acid functionalised polymeric membranes, Catal. Today. 118 (2006) 166–171.

[50] J. Saleh, A.Y. Tremblay, M.A. Dubé, Glycerol removal from biodiesel using membrane separation technology, Fuel. 89 (2010) 2260–2266.

[51] M.A. Dubé, A.Y. Tremblay, J. Liu, Biodiesel production using a membrane reactor, Bioresour. Technol. 98 (2007) 639–647.

[52] P. Cao, M.A. Dubé, A.Y. Tremblay, Methanol recycling in the production of biodiesel in a membrane reactor, Fuel. 87 (2008) 825–833.

[53] G.J. Harmsen, Reactive distillation: The front-runner of industrial process intensification, Chem. Eng. Process. Process Intensif. 46 (2007) 774–780.

[54] B. B. He, A. P. Singh, J. C. Thompson, A Novel Continuous-Flow Reactor Using Reactive Distillation for Biodiesel Production, Trans. ASABE. 49 (2006) 107–112.

[55] B. B. He, A. P. Singh, J. C. Thompson, Experimental Optimization of A Continuous-Flow Reactive Distillation Reactor for Biodiesel Production, Trans. ASAE. 48 (2005) 2237–2243.

[56] L. Simasatitkul, P. Siricharnsakunchai, Y. Patcharavorachot, S. Assabumrungrat, A. Arpornwichanop, Reactive distillation for biodiesel production from soybean oil, Korean J. Chem. Eng. 28 (2011) 649–655.

[57] Kusmiyati, A. Sugiharto, Production of Biodiesel from Oleic Acid and Methanol by Reactive Distillation, Bull. Chem. React. Eng. Catal. 5 (2010) 1–6.

[58] T. Poddar, A. Jagannath, A. Almansoori, Biodiesel Production using Reactive Distillation: A Comparative Simulation Study, in: Energy Procedia, Elsevier, 2015: pp. 17–22.

[59] D.H. Meikrantz, L.L. Macaluso, H.W. Sams, C.H. Schardin, A.G. Federici, Centrifugal separator, (1998).

[60] B. Schuur, J. Floure, A.J. Hallett, J.G.M. Winkelman, J.G. DeVries, H.J. Heeres, Continuous Chiral Separation of Amino Acid Derivatives by Enantioselective Liquid-Liquid Extraction in Centrifugal Contactor Separators, Org. Process Res. Dev. 12 (2008) 950–955.

[61] G.N. Kraai, B. Schuur, F. van Zwol, H.H. van de Bovenkamp, H.J. Heeres, Novel highly integrated biodiesel production technology in a centrifugal contactor separator device, Chem. Eng. J. 154 (2009) 384–389.

[62] M.Y. Abduh, W. van Ulden, V. Kalpoe, H.H. van de Bovenkamp, R. Manurung, H.J. Heeres, Biodiesel synthesis from Jatropha curcas L. oil and ethanol in a continuous centrifugal contactor separator, Eur. J. Lipid Sci. Technol. 115 (2013) 123–131.

[63] M.Y. Abduh, W. van Ulden, H.H. van de Bovenkamp, T. Buntara, F. Picchioni, R. Manurung, et al., Synthesis and refining of sunflower biodiesel in a cascade of continuous centrifugal contactor separators, Eur. J. Lipid Sci. Technol. 117 (2015) 242–254.

[64] M.Y. Abduh, A.F. Martínez, A. Kloekhorst, R. Manurung, H.J. Heeres, Experimental and modelling studies on continuous synthesis and refining of biodiesel in a dedicated bench scale unit using centrifugal contactor separator technology, Eur. J. Lipid Sci. Technol. 118 (2016) 938–948.

[65] I.M. Atadashi, M.K. Aroua, A.R. Abdul Aziz, N.M.N. Sulaiman, The effects of catalysts in biodiesel production: A review, J. Ind. Eng. Chem. 19 (2013) 14–26.

[66] A. Guldhe, B. Singh, T. Mutanda, K. Permaul, F. Bux, Advances in synthesis of biodiesel via enzyme catalysis: Novel and sustainable approaches, Renew. Sustain. Energy Rev. 41 (2015) 1447–1464.

[67] L.P. Christopher, Hemanathan Kumar, V.P. Zambare, Enzymatic biodiesel: Challenges and opportunities, Appl. Energy. 119 (2014) 497–520.

[68] K.E. Jaeger, M.T. Reetz, Microbial lipases form versatile tools for biotechnology, Trends Biotechnol. 16 (1998) 396–403.

[69] S. Shah, S. Sharma, M.N. Gupta, Enzymatic transesterification for biodiesel production, Indian J. Biochem. Biophys. 40 (2003) 392–399.

[70] S. Sellappan, C.C. Akoh, Applications of lipases in modifications of food lipids., in: C.T. Hou (Ed.), Handb. Ind. Biocatal., CRC Press LLC, Boca Raton, 2005: pp. 1–39.

[71] P.M. Nielsen, J. Brask, L. Fjerbaek, Enzymatic biodiesel production: Technical and economical considerations, Eur. J. Lipid Sci. Technol. 110 (2008) 692–700.

[72] A. Toftgaard Pedersen, M. Nordblad, P.M. Nielsen, J.M. Woodley, Batch production of FAEE-biodiesel using a liquid lipase formulation, J. Mol. Catal. B Enzym. 105 (2014) 89–94.

[73] J. Price, B. Hofmann, V.T.L. Silva, M. Nordblad, J.M. Woodley, J.K. Huusom, Mechanistic modeling of biodiesel production using a liquid lipase formulation., Biotechnol. Prog. 30 (2014) 1277–90.

[74] J. Price, M. Nordblad, J.M. Woodley, J.K. Huusom, Fed-Batch Feeding Strategies for Enzymatic Biodiesel Production, in: B. Edward (Ed.), IFAC Proc. Vol., 2014: pp. 6204–6209.

[75] P.M. Nielsen, A. Rancke-Madsen, H.C. Holm, R. Burton, Production of Biodiesel Using Liquid Lipase Formulations, J. Am. Oil Chem. Soc. 93 (2016) 3–8.

[76] J.K. Poppe, R. Fernandez-Lafuente, R.C. Rodrigues, M.A.Z. Ayub, Enzymatic reactors for biodiesel synthesis: Present status and future prospects., Biotechnol. Adv. 33 (2015) 511–25.

[77] J. Price, M. Nordblad, J.M. Woodley, From Fed-batch to Continuous Enzymatic Biodiesel Production, Comput. Aided Chem. Eng. 37 (2015) 1337–1342.

[78] J. Price, M. Nordblad, H.H. Martel, B. Chrabas, H. Wang, P.M. Nielsen, et al., Scale-up of industrial biodiesel production to 40 m(3) using a liquid lipase formulation., Biotechnol. Bioeng. (2016).

[79] K.R. Jegannathan, S. Abang, D. Poncelet, E.S. Chan, P. Ravindra, Production of biodiesel using immobilized lipase - a critical review., Crit. Rev. Biotechnol. 28 (2008) 253–264.

[80] X. Zhao, F. Qi, C. Yuan, W. Du, D. Liu, Lipase-catalyzed process for biodiesel production: Enzyme immobilization, process simulation and optimization, Renew. Sustain. Energy Rev. 44 (2015) 182–197.

[81] T. Tan, J. Lu, K. Nie, L. Deng, F. Wang, Biodiesel production with immobilized lipase: A review., Biotechnol. Adv. 28 (2010) 628–34.

[82] T. Tan, K. Nie, F. Wang, Production of Biodiesel by Immobilized Candida sp. Lipase at High Water Content, Appl. Biochem. Biotechnol. 128 (2006) 109–116.

[83] M. Babaki, M. Yousefi, Z. Habibi, M. Mohammadi, P. Yousefi, J. Mohammadi, et al., Enzymatic production of biodiesel using lipases immobilized on silica nanoparticles as highly reusable biocatalysts: effect of water, t-butanol and blue silica gel contents, Renew. Energy. 91 (2016) 196–206.

[84] J.H. Sim, A. Harun @ Kamaruddin, S. Bhatia, Effect of Mass Transfer and Enzyme Loading on the Biodiesel Yield and Reaction Rate in the Enzymatic Transesterification of Crude Palm Oil, Energy & Fuels. 23 (2009) 4651–4658.

[85] M. Iso, B. Chen, M. Eguchi, T. Kudo, S. Shrestha, Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase, J. Mol. Catal. B Enzym. 16 (2001) 53–58.

[86] J. Lu, Y. Chen, F. Wang, T. Tan, Effect of water on methanolysis of glycerol trioleate catalyzed by immobilized lipase Candida sp. 99–125 in organic solvent system, J. Mol. Catal. B Enzym. 56 (2009) 122–125.

[87] W. Chulalaksananukul, J.S. Condoret, P. Delorme, R.M. Willemot, Kinetic study of esterification by immobilized lipase in n -hexane, FEBS Lett. 276 (1990) 181–184.

[88] A.L. Paiva, V.M. Balcão, F.X. Malcata, Kinetics and mechanisms of reactions catalyzed by immobilized lipases, Enzyme Microb. Technol. 27 (2000) 187–204.

[89] A.C. Oliveira, M.F. Rosa, M.R. Aires-Barros, J.M.S. Cabral, Enzymatic esterification of ethanol and oleic acid — a kinetic study, J. Mol. Catal. B Enzym. 11 (2001) 999–1005.

[90] B. Cheirsilp, A. H-Kittikun, S. Limkatanyu, Impact of transesterification mechanisms on the kinetic modeling of biodiesel production by immobilized lipase, Biochem. Eng. J. 42 (2008) 261–269.

[91] P.S. Keng, M. Basri, A.B. Ariff, M.B. Abdul Rahman, R.N.Z. Abdul Rahman, A.B. Salleh, Scale-up synthesis of lipase-catalyzed palm esters in stirred-tank reactor, Bioresour. Technol. 99 (2008) 6097–6104.

[92] E.C.G.G. Aguieiras, E.D. Cavalcanti-Oliveira, D.M.G.G. Freire, Current status and new developments of biodiesel production using fungal lipases, Fuel. 159 (2015) 52–67.

[93] F.A.S. Fonseca, J.A. Vidal-Vieira, S.P. Ravagnani, Transesterification of vegetable oils: Simulating the replacement of batch reactors with continuous reactors., Bioresour. Technol. 101 (2010) 8151–7.

[94] J.-W. Chen, W.-T. Wu, Regeneration of immobilized Candida antarctica lipase for transesterification., J. Biosci. Bioeng. 95 (2003) 466–469.

[95] S. Chattopadhyay, R. Sen, Development of a novel integrated continuous reactor system for biocatalytic production of biodiesel, Bioresour. Technol. 147 (2013) 395–400.

[96] S. Ramachandran, S.K. Singh, C. Larroche, C.R. Soccol, A. Pandey, Oil cakes and their biotechnological applications – A review, Bioresour. Technol. 98 (2007) 2000–2009.

[97] W. Apiwatanapiwat, P. Vaithanomsat, P. Somkliang, T. Malapant, Optimization of Protein Hydrolysate Production Process from Jatropha curcas Cake, Eng. Technol. (2009) 109–112.

[98] A.S. Silitonga, A.E. Atabani, T.M.I. Mahlia, H.H. Masjuki, I.A. Badruddin, S. Mekhilef, A review on prospect of Jatropha curcas for biodiesel in Indonesia, Renew. Sustain. Energy Rev. 15 (2011) 3733–3756.

[99] Widyarani, E. Ratnaningsih, J.P.M. Sanders, M.E. Bruins, Biorefinery methods for separation of protein and oil fractions from rubber seed kernel, Ind. Crops Prod. 62 (2014) 323–332.

[100] F.S. Navarro-Pineda, S.A. Baz-Rodríguez, R. Handler, J.C. Sacramento-Rivero, Advances on the processing of Jatropha curcas towards a whole-crop biorefinery, Renew. Sustain. Energy Rev. 54 (2016) 247–269.

[101] U. Hölker, M. Höfer, J. Lenz, Biotechnological advantages of laboratory-scale solid-state fermentation with fungi, Appl. Microbiol. Biotechnol. 64 (2004) 175–186.

[102] A. Pandey, C.R. Soccol, D. Mitchell, New developments in solid state fermentation: I-bioprocesses and products, Process Biochem. 35 (2000) 1153–1169.

[103] U. Hölker, J. Lenz, Solid-state fermentation — are there any biotechnological advantages?, Curr. Opin. Microbiol. 8 (2005) 301–306.